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INTESTINAL ABSORPTION AND TRANSPORT IN *THYONE*.[1] II. OBSERVATIONS ON SUGAR TRANSPORT

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The biological background for the study of intestinal absorption and transport in *Thyone* was presented in the previous paper (Farmanfarmaian, 1969). This animal has a well developed intestine with tall mucosal epithelial cells. The luminal border of these cells is invested with numerous "microvilli" which are similar to the mammalian intestinal microvilli in size and structure and present an extensive surface for the absorption of nutrients.

A large part of the natural diet of the animal consists of plant particles which contain a variety of sugars. Glucose appears to be the most important sugar in the economy of *Thyone* since it constitutes half of the total dissolved carbohydrates of the natural gut fluid. Free glucose is also found in the intestinal tissue and the perivisceral fluid, the circulatory fluid of this animal. It is therefore the first sugar of choice for studying the intestinal transport of sugars in *Thyone*. The results of investigations of some of the physiological conditions which affect the *in vitro* and *in vivo* absorption, transmural transfer, and distribution of glucose are presented in this paper.

Materials and Methods

Animals and tissues

Procedures for the collection, maintenance and dissection of *Thyone* were given in the previous paper (Farmanfarmaian, 1969). For *in vitro* experiments, either the test tube method of Crane and Wilson (1958) or the everted sac method of Wilson and Wiseman (1954) were employed. These methods were slightly modified for the fragile intestine of *Thyone*. For the test tube method, tubes were made from 10 ml pipets with a funneled opening. In these experiments air was bubbled through a fine polyethylene tubing and the gut was suspended from a glass cannula attached to the arm of a micromanipulator. Everted sacs were incubated in Warburg flasks or in open beakers on a gyrotory shaker bath. All intestinal preparations were checked for leaks before and after each experiment and the leaky ones were discarded. In a few experiments leaks were detected by adding enough phenol red to the solution within the sac to make it just visibly colored. Leakage from such sacs into the incubation medium is conveniently observed. When samples of solutions containing phenol red were analyzed, adequate blanks or quench controls were used. In most experiments, however, each ligated sac

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was placed upon a piece of dry parafilm and its ends alternately lifted to create positive pressure within the sac. Under these conditions, the jet of colorless fluid issuing from leaky preparations in easily detected and the preparation discarded. Unless otherwise indicated, all the experiments were conducted at $20 \pm 0.5°$ C in an air atmosphere and all the solutions were made in isosmotic filtered sea water (FSW) at pH 7.9 ± 0.1. Specific conditions of each experiment are presented with the results.

Chemical analyses

Procedures for glucose analysis and thin layer chromatography (TLC) of sugars were described in the previous paper. The purity of glucose was checked by TLC.

The amaranth dye dilution method was used to measure changes in the water content of sacs. For this purpose 0.5 m*M* solutions of amaranth FSW were employed. Samples of 100 μl were obtained at the beginning and at the end of an experiment. After adding 3 ml FSW to these samples the $O.D._{520}$ was read against a 3.1 FSW blank in the Bausch and Lomb Spectronic 20 colorimeter.

All analyses were in two or more replicates.

Radiotracer procedures

The Packard Liquid Scintillation Spectrometer Model 3375 and radiochromatogram scanner Model 7201 were used for measurements of radioactivity. The procedures employed in the use of these instruments were generally in accordance with those described by Wang and Willis (1965) and the operation manuals provided with these instruments. Radioactive materials were purchased from New England Nuclear Corporation of Boston and the purity of these compounds were checked by TLC visually and by radiochromatographic scanning. Volumes of radioactive samples counted were 10–100 μl as desired. Two or more replicate samples were counted and appropriate blanks, quench controls and standards were included. To each scintillation vial 15 ml of Bray's scintillation fluid was added one hour before the counting. The composition of Bray's fluid was as follows: Napthalene 60 g, Methanol 100 ml, Ethylene Glycol 20 ml, "PPO" 4 g, "POPOP" 200 mg, and enough P-Dioxane to make one liter of fluid. Scintillation chemicals were purchased from the Packard Instrument Company, Downers Grove, Illinois.

Treatment of data

Computations related to the radiotracer method were in accordance with Wang and Willis (1965) or the operation manual of the instruments. Statistical treatment was generally in conformity with those described by Fisher (1958).

Validation of the radiotracer method

In transport studies the radionuclide incorporated into a compound under investigation may appear in other compounds due to exchange or metabolic conversions. Accordingly, quantitative changes in radioactivity may not reflect a proportional change in the quantity of the compound under study. It is therefore necessary to validiate the radiotracer method for each compound and experimental

TABLE I

*Validation of the radiotracer method**

Incubation medium C^{14}-glucose concentration mM	Series A		Series B	
	Glucose oxidase method	Radiotracer method	Glucose oxidase method	Radiotracer method
	$\frac{\mu g \text{ absorbed}}{\mu g \text{ in initial solution}}$	$\frac{\text{CPM absorbed}}{\text{CPM in initial solution}}$	$\frac{\mu g \text{ absorbed}}{\mu g \text{ in initial solution}}$	$\frac{\text{CPM absorbed}}{\text{CPM in initial solution}}$
2	$\frac{3.5}{17.5} = 0.20$	$\frac{5975}{31384} = 0.19$	$\frac{7.2}{16.7} = 0.43$	$\frac{12905}{29485} = 0.44$
4	$\frac{1.1}{14.8} = 0.07$	$\frac{1113}{12476} = 0.08$	$\frac{3}{13} = 0.23$	$\frac{2687}{11962} = 0.22$
6	$\frac{1.0}{21.0} = 0.05$	$\frac{786}{13398} = 0.06$	$\frac{3.7}{20.5} = 0.18$	$\frac{1910}{13028} = 0.15$

* Conditions of experiments: Series A—everted sac, proximal clear zone 0.0457 g wet. Series B—everted sac, distal clear zone 0.1564 g wet. Each sac was first incubated in the 2 mM solution, then washed in FSW and reincubated in 4 and then in 6 mM solutions in Warburg flasks. Incubation medium, 1.5 ml of various glucose solutions FSW; serosal medium 0.3 ml FSW; incubation time in each solution 30 minutes. Sample size 50 μl at 2 mM and 20 μl at 4 and 6 mM.

procedure used. This may be done by radiochromatography or by chemical quantitative methods. The latter method was used under actual experimental conditions to validate the radiotracer procedure adopted for the studies on glucose absorption.

Everted sacs were incubated in different concentrations of C^{14} glucose and the absorption of glucose was determined as the difference between initial and final concentration of the incubation medium. Duplicate samples were analyzed by both glucose oxidase and the radiotracer methods. The results are expressed in Table I as the ratio of μg absorbed to μg in the intial solution, and CPM absorbed to CPM in the initial solution. These ratios are practically identical for each concentration indicating that the radiotracer method faithfully measures the quantity of glucose absorbed. These experiments also demonstrate that under the prescribed conditions, it is not necessary to treat the samples with TCA prior to analysis. An appreciable breakdown of glucose due to enzymes released into the medium would have caused a difference in the corresponding ratios of the two methods. The validity of the radiotracer method for all the experiments reported in this paper was established either by radiochromatography or by procedures similar to those described above.

RESULTS

Physiological medium and pH

The perivisceral fluid of holothurians is similar to the surrounding sea water in ionic composition and osmotic pressure (Farmanfarmaian, 1969). Since the animal is a particle feeder, presumably some sea water enters the digestive tract along with the food particles.

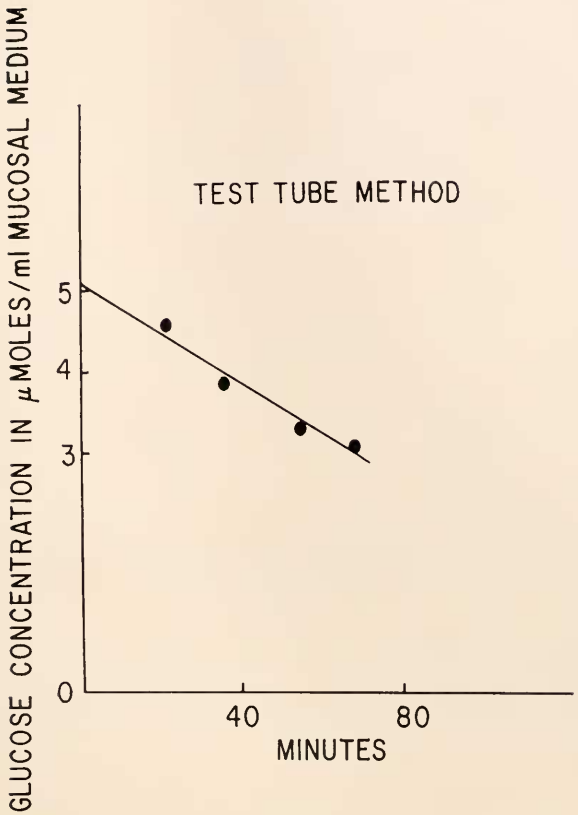

FIGURE 1. Reduction of D-glucose concentration in the mucosal medium due to absorption by the mucosal epithelium. Conditions of the experiment: Clear zone segment in test tube; mucosal medium 0.5 ml of 5 m*M* glucose FSW solution; serosal medium 4 ml of FSW.

These initial considerations prompted the use of filtered sea water as the physiological medium in which transport studies were carried out. Sea water has an appreciable amount of buffer capacity at pH 8.0 (Sverdrup, Johnson, and Fleming, 1942). Titration of filtered sea water used in the reported experiments gave a buffer capacity equivalent to 0.1 unit decrease in pH when 7 cc of N/1000 HCl was added to 100 cc of filtered sea water at pH 7.9.

In a series of experiments where 0.1–0.2 g of fresh tissue and 1.5–4 ml of unbuffered filtered sea water glucose solutions were used, the pH of the medium at the start of the experiment ranged between 7.7–8.0 and at the end of 1–2 hours incubation, 7.5–7.8. During this time glucose absorption was essentially linear (Figs. 1 and 2). These experiments showed that during long incubations the pH may drop by as much as 0.3 units but this pH change has no appreciable effect on glucose absorption in the range of 7.5–8.0. In experiments of short duration (< 20 minutes), the pH did not change. The effect of pH on glucose absorption in the range of 7.8–9.0 were studied using Tris buffered solutions of glucose in filtered sea water. The results presented in Table II show that there is a small

TABLE II

*Effect of pH on the absorption of C^{14}-glucose**

Tissue wet weight in g	pH	Initial CPM/100 µl	Final CPM/100 µl	CPM absorbed / CPM in initial solution	As per cent of pH 7.8	Per cent change
0.1837	7.8	7590	6020	0.207	100	—
	8.2	12030	9300	0.227	110	+10
	8.6	10450	8140	0.221	107	+7
	9.0	17490	13680	0.218	105	+5
0.1712	7.8	7500	6150	0.180	100	—
	8.2	12080	9510	0.213	118	+18
	8.6	10490	8260	0.212	118	+18
	9.0	17450	14020	0.196	109	+9
0.1309	7.8	7500	6260	0.165	100	—
	8.2	12080	9320	0.228	138	+38
	8.6	10490	8550	0.185	112	+12
	9.0	17450	14020	0.196	119	+19

* Conditions of experiment: First loop everted sacs in open beakers. Each sac was first incubated for 5 minutes in pH 7.8 solution, then washed in FSW and reincubated for 5 minutes in the next solution and so on. Incubation medium, 2 ml of 0.5 m*M* C^{14}-Glucose FSW buffered with 5 m*M* Tris at the given pH. Serosal medium, 0.9 ml of FSW.

stimulation of absorption at higher pH. In all cases maximum absorption was at pH 8.2. A few measurements of the pH of the intestine were made by applying the electrode to the mucosal epithelium directly. The results were between 7.6 and 8.0.

TABLE III

*Absorption of C^{14}-glucose from filtered sea water (FSW) and centrifuged perivisceral fluid (PF) solutions**

Tissue wet weight in g	Paired test solutions 1.5 ml	CPM absorbed / CPM in initial solution	Per cent difference
0.1693	0.25 m*M* glucose FSW	$\frac{5646}{8128} = 0.69$	10
	0.25 m*M* glucose PF	$\frac{2829}{4579} = 0.62$	
0.1522	0.50 m*M* glucose FSW	$\frac{2314}{8963} = 0.26$	0
	0.50 m*M* glucose PF	$\frac{25466}{97038} = 0.26$	

* Conditions of experiment: First loop everted sacs in Warburg flasks. Each sac was incubated for 10 minutes in the first solution, then washed in FSW and reincubated in the second solution for 10 minutes. pH of centrifuged perivisceral fluid was 7.5 and that of filtered sea water 8.0. Intestine and perivisceral fluid of the same animal was used. Serosal medium, 0.3 ml of FSW or PF.

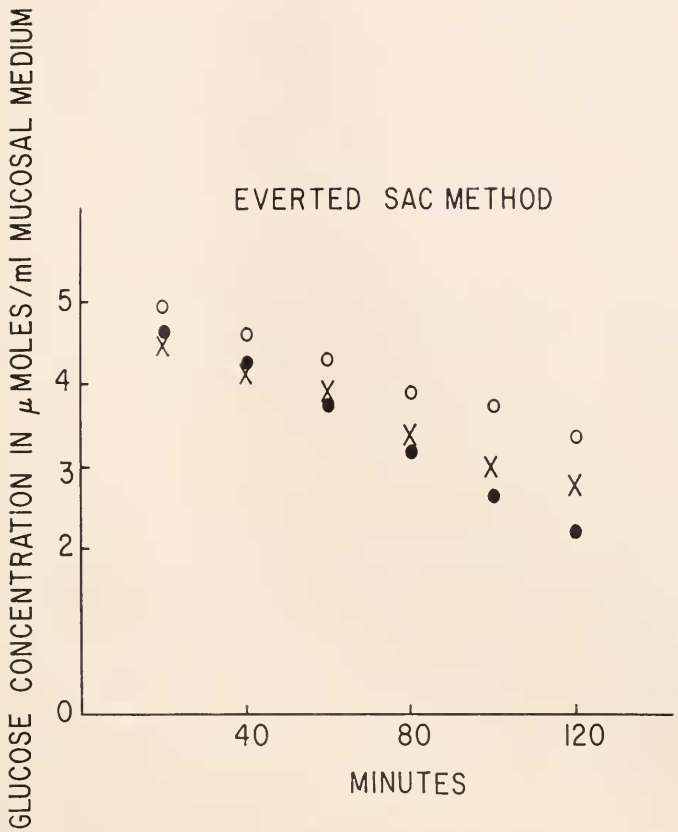

FIGURE 2. Reduction of D-glucose concentration in the mucosal medium due to absorption by the mucosal epithelium. Conditions of the experiment: Everted sacs of clear zone from three different animals; in Warburg flasks; mucosal medium 1.5 ml of 5 m*M* glucose FSW solution; serosal medium 0.6 ml FSW.

In two experiments the absorption of glucose from solutions in filtered sea water and from solutions in centrifuged perivisceral fluid was compared. The results are given in Table III. These results indicate that there is no appreciable difference between glucose absorption from filtered sea water solutions and from centrifuged perivisceral fluid solutions. Ferguson (1964) also reported no difference in glucose uptake by the digestive gland of *Asterias* when incubated in filtered sea water or centrifuged perivisceral fluid.

On the basis of the above considerations filtered sea water was chosen as the physiological medium and buffered with Tris at pH 7.9 ± 0.1 when necessary.

Net water flux

The net flux of water occurring simultaneously with glucose absorption was studied by amaranth dye dilution, using the test tube method. Samples taken at approximately 20 minute intervals through the gut cannula showed a linear glucose

Table IV

*Net water flux during the absorption of glucose**

Intestinal region and dry weight	Experiment duration in hours	% of total glucose absorbed	OD_{520} for Amaranth	
			Initial	Final
Clear zone 0.0155 g	1.5	80	0.32	0.31
Cloacal segment 0.0156 g	2.0	40	0.29	0.30

* Conditions of experiment: Test tube method; mucosal medium 0.5 ml of 5 m*M* glucose and 0.5 m*M* Amaranth FSW; serosal medium 4 ml of FSW. After introduction of mucosal medium, the fluid level in the lumen was raised and lowered several times in order to mix the dye solution with residual fluid. Then 100 μl of solution was removed through the glass cannula as the initial sample and similarly at the end of the experiment as the final sample. OD_{520} was determined by adding 3 ml FSW to each sample and reading against a 3.1 ml FSW blank.

absorption rate similar to that in Figure 1. There was no change in the concentration of the dye during a 1.5 and a 2 hour incubation period. The terminal data are summarized in Table IV. These results show that while 80% and 40% of the total glucose was absorbed by the mucosal epithelium of the clear zone and cloacal segments, respectively, there was no appreciable net water flux. In experiments with the everted sac method reported in Figure 2, 0.60 ml of fluid was placed within each sac by means of a syringe graduated to 0.01 cc. After a three hour incubation, the fluid content of the sac was collected on a piece of dry parafilm and measured in the same type of syringe. The final volume was within $\pm$ 0.05 ml of the original volume indicating no appreciable net water flux. Such observations have been reconfirmed in many experiments where transmural transport was measured.

Effect of captivity

The anterior parts of the digestive tract of freshly collected specimens of *Thyone* contain a large amount of particulate matter. In the laboratory the first loop and the clear zone are usually empty after a few days. It must be assumed, therefore, that the animals undergo partial starvation in captivity. For this

Table V

*Effect of captivity period on C^{14}-glucose absorption rate**

No. days in lab.	Absorption μmoles/min/ml tissue H_2O $\pm$ SEM
1–4	0.72 $\pm$ 0.19
12	0.38 $\pm$ 0.10
26	0.36 $\pm$ 0.08
32	0.34 $\pm$ 0.07

* Conditions of experiment: Everted sacs of the first loop; open beakers in gyrotory shaker; incubation medium 2 ml of 1 m*M* glucose isotonic FSW; serosal medium 0.3 ml FSW; incubation time 5 minutes; standard error of the mean (SEM) is for $n = 3$ and $P < 0.1$.

TABLE VI

Relative absorption of glucose by everted segments from various regions of Thyone *intestine**

Animal number	First loop	Clear zone	Second loop	Cloacal segment
1	100	100	—	15
2	100	100	28	42
3	100	110	35	63
4	100	78	—	50
5	100	106	21	14
6	100	108	44	45

* Conditions of experiments; 1.5 ml of m*M* glucose FSW in Warburg flasks; serosal medium 0.3 ml FSW; incubation time 1 hour.

reason the effect of captivity on glucose absorpion by everted sacs from the first loop was investigated. The results reported in Table V show that by the 12th day after collection, glucose absorption is reduced to 50% of the values observed for animals which had been in captivity from 1–4 days. This reduction stabilizes so that the extension of the captivity period to as long as 32 days did not cause further appreciable reduction in the capacity to absorb glucose. These data further indicate that it is best to restrict studies on transport rates to the first few days after collection when the velocities are higher. In experiments which are designed to yield comparable results, animals of similar background and preferably from the same collection should be used. These guidelines have been followed in the present studies.

Regional capacity for absorption

The different regions of the intestine of *Thyone* were anatomically defined in the previous paper. In a series of experiments, the capacity for the absorption of glucose by each region was measured and the results expressed relative to the value obtained for the first loop region (Table VI). When the different intestinal regions from the same animal are compared, the first loop and the clear zone have the highest absorptive capacities and are nearly equal in this respect. These regions appear analogous to the jejunum and the ileum of the mammals. The second loop and the cloacal segment generally show less than half the absorptive capacity of the first loop. The cloacal segment appears to have a higher capacity than the second loop in some cases, but this is probably due to greater glucose utilization; this part of the intestine is more heavily muscularized and exhibits strong tonic and rhythmic contractions when distended.

In vivo *absorption and distribution of* C^{14}*-glucose*

A series of experiments were carried out in order to determine the region of intestinal absorption and the distribution of labeled glucose under *in vivo* conditions. These experiments provided additional information about the relative role of the hemal sinuses and the perivisceral fluid in the distribution of the absorbed sugar (Farmanfarmaian, 1969). The procedures were as follows: The animal was relaxed, washed, dissected, and immobilized in a wax bottomed finger

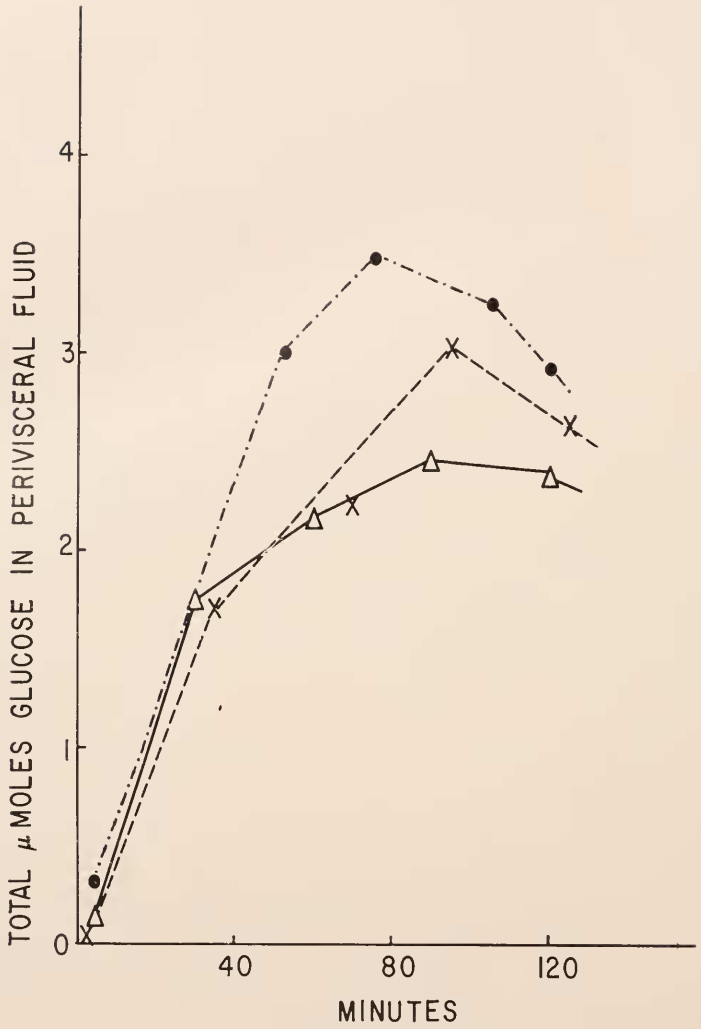

FIGURE 3. Transmural transport of D-glucose *in vivo* for three different animals. For conditions of the experiments see text.

bowl as previously described. The finger bowl was tilted and all the fluid drained. Then the animal was covered with 100 ml of filtered sea water which acts as a substitute for the perivisceral fluid. A small aerator bubbling air into the fluid, simultaneously provided aeration and mixing. The whole preparation was placed in a 20° ± 0.5° C bath and 0.5 ml of 0.5 m*M* glucose C^{14} and 0.5 m*M* amaranth in filtered sea water was intubated via the cannulated oropharynx directly into the stomach. The bathing fluid was sampled at hourly intervals. The duration of experiment was 5 hours and during this period peristalsis of the gut, circulation of the red hemocytes in the ampullae of the podia, and the movements of the branches of the water lungs were readily observable, but no circulation could be seen in the

TABLE VII

Distribution of C^{14} activity after intubation of C^{14}-glucose solution into the stomach of Thyone *under* in vivo *conditions**

Animal number		1	2
Total CPM intubated into the stomach		842250	847850
Total CPM recovered**		7575	6975
Total CPM absorbed		834675	840875
Per cent CPM absorbed		99%	99%
Total CPM in 100 ml substituted Perivisceral fluid at hourly intervals			
	hour 1	5000	3500
	hour 2	19500	25000
	hour 3	27000	28000
	hour 4	25000	21000
	hour 5	25000	30000
Per cent CPM transmural transport at the end of 3rd hour***		3.2%	3.4%
Total CPM stored in stomach and first loop tissues		807675	810875
Per cent CPM stored in stomach and first loop tissues		96.8%	96.5%
CPM/mg drained wet weight of tissue			
Stomach		85–100	150–179
Anterior first loop		816–1419	771–908
Posterior first loop		281–598	79–189
Sinuses of hemal network with contents		6	7
Clear zone		1–2	1–2
Second loop		1–2	1–2

* For conditions of the experiment see text.

** This is total activity recovered when at the end of 5 hours the posterior end of the first loop is sectioned and all the lumen fluid in the loop collected. Volume change was corrected by the Amaranth dye dilution method.

*** CPM absorbed by other tissues from the bathing fluid is not taken into account.

hemal sinuses even though these were carefully examined under the dissecting scope. The reddish-colored intubated solution reached the end of the first loop at 4.5 to 5 hours. At the end of the experiment the bathing fluid was pipeted out; the posterior terminus of the first loop was sectioned, and all the fluid from the lumen of this region of the intestine was collected. The total radioactivity in this fluid was determined after the appropriate quench corrections and the volume correction by the amaranth dye dilution method.

Most of the hemal sinuses forming the network between the first loop and the second loop were tied off in a bundle at both ends and severed beyond the ligatures. In this manner these sinuses and their contents were removed, drained of their external water on filter paper and weighed. This material was loosely spread at the bottom of a scintillation flask, Bray's scintillation fluid added, and counted. Four or more tissue samples, each measuring 0.5 cm^2, were obtained from the different regions of the digestive tract. These were similarly blotted, weighed and spread at the bottom of the scintillation flasks for counting. Tissues counted in this manner are not quench corrected and therefore provide only an estimate of the activity distribution within the tissues.

Such experiments were performed on four animals with good reproducibility. The results for two animals presented in Table VII show that 99% of the glucose

was absorbed before the intubated solution reached the end of the first loop. By the end of the third hour about 3.3% of the absorbed sugar was in the substituted perivisceral fluid. The rest of the activity was stored mainly in the tissues of the first loop, particularly in the anterior part. This confirms the results of the *in vitro* experiments which showed that the first loop and clear zone are the main regions of intestinal absorption.

Although the sinuses of the hemal network are directly connected to the first loop (region of highest activity), there was no appreciable amount of radioactivity within these sinuses. By contrast, appreciable amounts of radioactivity could be recorded from the substitute perivisceral fluid at every sample interval and a peak was attained by the third hour. The magnitude of the activity in this fluid may have been 10× higher if the volume of the substitute fluid had been equal to the volume of the normal perivisceral fluid, about 10 ml. Unfortunately such an arrangement is not possible because under the experimental conditions a larger volume is necessary to cover the animal in the dish.

The amount of radioactivity in the substitute fluid was easily measurable but the concentration of glucose in 1 ml samples was below the sensitivity of the glucose oxidase method. This was due to the intubation of a meager quantity (45 μg) of high specific activity glucose so as to simulate the concentration in the natural gut fluid. In order to measure the transmural transfer of glucose by the glucose oxidase method *in vivo,* similar experiments were carried out after intubation of 2 ml of 10 m*M* glucose solution into the stomach and the restriction of the substitute fluid to 35 ml. One ml samples were removed at 30 minute intervals for 2 hours. These samples were directly analyzed by the purified glucose oxidase (Glucostat Special). Figure 3 illustrates the results obtained from 3 animals. These curves corroborate the isotope studies and show that glucose is rapidly transferred from the lumen to the substitute perivisceral fluid where it attains a peak in 1.5 hours. In these experiments the peak was attained in a shorter time because a larger volume (2 ml) of solution was intubated and as a result the glucose solution rapidly spread into the first loop. Of the total intubated glucose, 88–90% was absorbed by the first loop and 12–17% of the same total was found in the substitute fluid at the peak concentration.

Discussion

The studies on the effect of pH show that within the normal range of pH(s) recorded for the surrounding sea water (about 8.0), the perivisceral fluid (7.3–7.8) and the intestinal mucosal surface (7.6–8.0), change in pH has little effect upon glucose absorption by the intestinal segments of *Thyone.* At higher pH, 8.2 and above, there is an appreciable stimulation of glucose absorption. Jackson, Levin, and Thompson (1968) reported that high pH (7.8) stimulated glucose metabolism in the rat but not the mucosal transfer of glucose. It is not clear whether the stimulation of absorption noted for *Thyone* is due to increased glucose metabolism which secondarily facilitates absorption or to a direct stimulation of mucosal absorption.

Absorption of glucose was not accompanied by any appreciable absorption or transfer of water is experiments where the initial mucosal and serosal solutions were isosmotic. In the mammalian intestine, active transfer of glucose across the

intestinal wall creates an osmotic gradient which results in a net transfer of water (Wilson, 1962; Smyth, 1965, 1968). In addition to this osmotic transfer, glucose and certain other sugars specificially stimulate the transfer of water *in vitro*. Barry, Smyth and Wright (1965) pointed out that fluid transfer stimulated by sugars is related to their hexokinase specificity, and therefore energy production, and not necessarily to their hexose accumulation-transport specificity as defined by Crane (1960). Glucose has both of the above specificities; therefore, in the rat intestine it is transported against a gradient and at the same time stimulates water absorpion. Galactose has only the Crane specificity and does not stimulate water absorption. Fructose has the hexokinase specificity only; it stimulates water absorption but is not transported against a gradient. Barry, Smyth and Wright (1965) concluded that fluid transfer across the intestinal wall of the rat depends on the total solute transfer. Sugars may contribute either by being a component of the solutes transferred or by providing the energy required for the transport of other solutes such as Na^+ (Schultz and Curran, 1968).

Simultaneous intestinal absorption of sugars and water have been studied in a few animals other than mammals. Fox (1961) reported that there was little water movement in either direction when intestinal segments of the turtle, *Chrysemys picta*, were incubated with different monosaccharides for one hour. Csáky and Thale (1960) and Musacchia, Neff and Westhoff (1964) reached the same conclusion for the toad, *Bufo bufo*, and the catfish, *Ictalurus nebulosus*. It is difficult to reconcile this lack of water movement with the observation of accumulation transport of sugars reported by the same authors. In the case of the bullfrog, *Rana catesbeina*, Lawrence (1963) states that net transfer of sugar is accompanied by net transfer of water.

Treherne (1967) reported that water uptake in the midgut and hindgut of several species of insects occurs both by osmosis from dilute solutions and by mechanisms linked to the active transport of Na^+. Rapid net absorption of water from amino acid solutions introduced into the midgut of the locust, *Schistocerca gregaria*, was observed by Treherne. He concluded that this was a mechanism by which the concentration of amino acids in the lumen is raised so that a downward diffusion gradient between the lumen and the haemolymph is established. Absorption of glycine and serine appears to be dependent upon the establishment of such a diffusion gradient.

For marine invertebrates, Lawrence, Lawrence, Greer and Mailman (1967) stated that there was no net movement of water, Na^+, or Cl across the gut of the holothurian *Stichopus parvimensis* during incubation with various sugar and amino acid solutions. This statement is in agreement with the data presented in Table IV for *Thyone*. In the amphineuran mollusk, *Cryptochiton stelleri*, studied by Lawrence and Lawrence (1967), there appears to be no net transport of water even though glucose is reported to be transported against gradient in the anterior intestine. It should be remembered that the body fluids of the three marine species mentioned above are closely similar to their environmental sea water in osmotic pressure and ionic composition. Furthermore, these species spend their entire life cycles in marine waters so that they do not normally face any water balance problems.

In summary, the above investigations of net water flux in the intestine of various

animal groups indicate that absorption of water is dependent upon total solutes transfer. Its magnitude is related to the overall water balance of the animal; thus, intestinal water transport is significant for mammals and insects but not for *Thyone* or the other semiaquatic and aquatic animals cited above.

Thyone undergoes partial starvation in captivity. This results in a reduction of glucose absorption by the intestine. Similar observations were recorded from *in vivo* experiments on fasting rats when the fast period was extended to 48 hours (Wiseman, 1964). On the other hand the *in vitro* studies of Kershaw, Neame and Thompson (1960) and Crane and Mandelstam (1960) indicated that sugar absorption either did not change or was stimulated when the fasting period was extended beyond 24 hours in rats or hamsters. These investigations do not clarify the mechanisms responsible for the effects of starvation on intestinal transport mechanisms.

The combined *in vitro* investigations of the regional capacity for glucose absorption and the *in vivo* studies presented above directly support the views proposed in the previous paper (Farmanfarmaian, 1969) and permit the following conclusions for *Thyone*.

The first loop or the anterior part of the intestine is the most significant region for the absorption of sugar. The clear zone which follows the first loop provides the reserve capacity for absorption required during continuous feeding. The second loop and cloacal segment of the intestine appear to be concerned with the final formation of feces.

Depending upon the concentrations of the sugar used and the duration of the experiment, 3–17% of the absorbed sugar is directly transported into the perivisceral fluid. The hemal sinuses do not have a significant role in this translocation. The balance of the absorbed sugar is stored in the intestinal tissue, the bulk of which is represented by the large mucosal epithelial cells. It is important to emphasize that the echinoids and holothurians do not possess organs such as pancreas, liver, or hepatic caeca (Farmanfarmaian and Phillips, 1962). Therefore, digestion, absorption, and the immediate storage of nutrients is entirely the function of the mucosal epithelial cells of the gut.

Further studies directly concerned with the mechanisms of absorption and transport of sugars in *Thyone* will be published in a later communication.

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Summary

In the first paper of this series the biological background for the study of absorption and transport of sugars in the intestine of *Thyone* was presented. In this paper, physiological parameters which affect the absorption of glucose such as the nature of the incubation medium, the effect of pH, the net flux of water, and the effect of starvation under captivity have been studied.

The capacity for glucose absorption by different regions of the intestine was investigated under *in vitro* and *in vivo* conditions. The first loop is the main site for the absorption and transport of sugar and the clear zone of the intestine provides the reserve capacity for absorption during continuous feeding.

The *in vivo* studies demonstrate that the intestinal tissue acts as the first storage site for the absorbed sugar. Only 3–17% of the glucose absorbed is rapidly transferred into the perivisceral fluid for distribution to other organs. The hemal sinuses are not effectively involved in the distribution of absorbed glucose. The perivisceral fluid is the functional circulatory fluid.

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